1994) in view of Shi *et al.* (J. Biol. Chem. 270:2212-2119, 1995).

In the October 1, 2002 Office Action, claims 2-12 and 15-18 were newly rejected under 35 U.S.C. 103(a) over Popot *et al.*, Hoflack *et al.*, and Teufel *et al.* (EMBO J. 12:3399-3408, 1993) in view of Okamoto *et al.* (Cell 67:723-730, 1991).

Applicants respectfully request reconsideration on the merits of this application.

Summary of rejections under 35 U.S.C. 103(a)

Claims 2-12 and 15-18 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Popot *et al.* (Current Opinion in Biotechnology 6:394-402, 1995), Hoflack *et al.* (Trends in Pharm. Sci. 15:7-9, 1994) and Teufel *et al.* (EMBO J. 12:3399-3408, 1993), in view of Okamoto *et al.* (Cell 67:723-730, 1991).

Popot *et al.* is cited as teaching: "chimeric constructs of bacteriorhodopsin and G-protein receptors can be made for the purposes of functional and structural investigations (p. 396, column 1)"; that bacteriorhodopsin can be used as a 'benchtop' to arrange engineered loops (p. 397, col. 2); and that a "wealth of data indicates that most of the six loops connecting the transmembrane helices in bacteriorhodopsin can be tampered with to large extents and at least three of them can be cut without preventing refolding of the proteins (e.g. cytoplasmic loop III, ref. 61)." The Examiner further asserts that "the use of archaebacterium for recombinant expression of bacteriorhodopsin chimeras is old and well established in the art, as disclosed by Popot *et al.* (p. 397, col. 2)."

Hoflack *et al.* is characterized as teaching that it is "old and well established that bacteriorhodopsin is famous as a template to construct three dimensional models of G-protein coupled receptors".

Teufel *et al.* is said to teach "that the protein architecture of bacteriorhodopsin (BR) 'suggests the possibility of using BR as a structural scaffold in the construction of biological membranes with new and pre-defined properties by replacing the extra-membrane parts of BR with exogenous polypeptide modules of known function', see col 2 of page 3399. Additionally Teufel *et al.* teach that the 'structural integrity of loops B/C, CD, D/E and E/F (E/F is the third cytoplasmic loop) is not a prerequisite of BR function and that the construction of multifunctional proteins on the basis of BR as a structural scaffold is a feasible proposition. Loops B/C, CD, D/E and E/F are now clearly identified as prime candidates for future constructions of more complex loop replacements' see the last paragraph of page 3405. Additionally, Teufel *et al.* define what residues are to be considered the third cytoplasmic loop, see Fig 1, which correspond exactly to

amino acids 171-179 of the instant SEQ ID NO:2."

Okamoto *et al.* is cited as teaching that "peptides corresponding to the third cytoplasmic loop of a GPRC, e.g. the human β-adrenergic receptor, can activate G-protein."

The Examiner concludes that it would have been obvious to "construct chimeric bacteriorhodopsin/GPCRs, as taught by Potpot *et al.* and Teufel *et al.* using regions that are structurally analogous between GPCRs and bacteriorhodopsin, as is well established in the art (see Hoflack *et al.*), particularly that of the third intracellular loop of the human β-adrenergic receptor as taught by Okamoto *et al.* The motivation to do so is provided by Popot *et al.* who teach that bacteriorhodopsin can be used as a 'bench top' on which to arrange engineered loops that are designed to form binding or catalytic site (p. 397, col 2) and by Okamoto *et al.* who teach the third intracellular loop of the human β-adrenergic receptor provides for binding and activation of G-proteins, and who also teach the need for further study of the structure and function of the third intracellular loop of the human β-adrenergic receptor as is well appreciated in the art, e.g. see Introduction and Discussion. Further, the construction of a bacteriorhodopsin chimera at amino acids 171-179 (intracellular loop III) is suggested by Teufel who show these residues to define the cytoplasmic loop III".

Applicants' response to rejections under 35 U.S.C. 103(a)

Applicants respectfully disagree with the Office Action's Examiner's characterization of the prior art and assert that a prima facie case of obviousness has not been established. A prima facie case of obviousness requires: (1) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) a reasonable expectation of success; and (3) the art reference or combination of references must teach all of the claim limitations (MPEP 2142). The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) (MPEP 2143).

Applicants maintain that the cited references do not combine to teach or suggest a chimeric fusion protein comprising bacteriorhodopsin in which substantially all of the intracellular loop 3 domain of bacteriorhodopsin is replaced by a portion of the intracellular loop 3 domain of a G protein-coupled receptor protein, let alone by a portion of the intracellular loop 3 domain of a human β-adrenergic receptor. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the

resultant modification. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990). Applicants respectfully submit that the combination of art does not suggest the desirability of making the claimed invention.

Applicants disagree with the Examiner's assertion that "Popot *et al.* teaches chimeric constructs of bacteriorhodopsin and G-protein receptors can be made for the purposes of functional and structural investigations (p. 396, column 1)." On the contrary, Applicants found no mention of chimeric constructs of bacteriorhodopsin and G-protein coupled receptors anywhere in the publication. At p. 395, column 2, G-protein-coupled receptors are discussed with regard to split protein experiments in which fragments of dissected α-helical membrane proteins, including G-protein coupled receptors, were reassociated. However, Popot *et al.* does not teach chimeric fusion proteins comprising sequences from bacteriorhodopsin and a G-protein coupled receptor.

Applicants were unable to find any basis for the Examiner's assertion that Popot *et al.* teaches "the use of archaebacterium for recombinant expression of bacteriorhodopsin chimeras is old and well established in the art (p. 397, col. 2)." In fact, there is no mention of "archaebacterium" in any context, let alone in the context of recombinant expression of bacteriorhodopsin chimeras. Applicants note, however, that Popot *et al.* does cite to the Teufel *et al.* publication, which was also cited by the Examiner. Teufel *et al.* disclose a recombinant chimera of bacteriorhodopsin including sequences from the archaebacteria *Halobacterium salinarium* expressed in *Eschericia coli* or *Schizosaccharomyces pombe* (Abstract, p. 3399, column 1). Neither *Eschericia coli* or *Schizosaccharomyces pombe* is an archaebacteria.

Teufel disclosed a chimeric protein in which a region of the intracellular loop 3 region of bacteriorhodopsin was replaced with a 13 amino acid residue C-terminal sequence of Sendai virus L-protein, which region the Examiner characterized as corresponding exactly with to amino acid residues 171-179 of SEQ ID NO:2, as shown in Fig. 1 of Teufel.

In contrast to Teufel *et al.*, which taught a chimeric protein in which a viral tridecapeptide was used to replace a portion of the intracellular loop 3 domain of bacteriorhodopsin, the claimed invention is drawn to a chimeric fusion protein comprising bacteriorhodopsin in which the intracellular loop three domain is replaced by at least a portion of the intracellualr loop 3 domain of a GPCR, which is a mammalian sequence. One skilled in the art would appreciate that the ability to obtain a chimeric fusion protein comprising archaebacterial and mammalian sequences could not be predicted from a chimeric fusion protein comprising archaebacterial and a viral tridecapeptide. Nor could one predict the expression of such a chimeric fusion protein in archaebacteria, as required by claim 11. As one of ordinary skill in the art would appreciate,

expression of such a bacteriorhodopsin chimeric fusion protein in which loop three was replaced with a mammalian GPCR loop three sequence could be expected to have deleterious effects on the host organism, making it difficult to obtain such proteins. Difficulty obtaining expression of a bacteriorhodopsin-GPCR chimeric fusion protein would be expected to increase when a portion of the bacteriorhodopsin (amino acid residues 171-179) is replaced with amino acid residues 12-73 of SEQ ID NO:47, which constitutes a relatively large increase in the size of the intracellular loop 3 domain, as required by claim 18.

Claim 3 requires that amino acid residues 171-179 of SEQ ID NO:2 be replaced by at least a portion of the intracellular loop 3 domain of a GPCR. Although Teufel *et al.* disclosed in Fig. 1 that the intracellular loop 3 domain of bacteriorhodopsin is defined by a sequence corresponding exactly with amino acid residues 171-179 of SEQ ID NO:2, in the chimeric protein of Teufel *et al.*, amino acids corresponding to amino acid residues 172-178 of SEQ ID NO:2 are replaced by the Sendai virus peptide sequence. One skilled in the art would not be motivated to modify the teachings of Teufel *et al.* to replace intracellular loop 3 amino acid residues corresponding to amino acids 171-179 of SEQ ID NO:2, as required by claim 3, rather than replacing residues 172-178, as taught by Teufel.

Although Teufel *et al.* teach that a monoclonal antibody directed against a 13 amino acid viral peptide comprised within a chimeric bacteriorhodopsin retains its function (i.e., its ability to bind to the viral peptide), and that 'the construction of multifunctional membrane proteins on the basis of BR as a structural scaffold is a feasible proposition', Teufel *et al.* provides no teaching as to which types of constructs would retain functionality. Furthermore, Teufel *et al.* provides no teaching to suggest that a chimeric fusion protein in which the intracellular loop 3 region of bacteriorhodopsin was replaced by the intracellular loop 3 region a GPCR would have the ability to alter or increase the rate of GTP-GDP exchange on a G protein *in vivo*, as required by claims 4 and 5. In view of the fact that the only function demonstrated was binding of a viral peptide to a monoclonal antibody, Applicants submit that the broad assertion 'the construction of multifunctional membrane proteins on the basis of BR as a structural scaffold is a feasible proposition' was a merely an invitation to experiment with any of an essentially unlimited number of potentially functional moieties, which may or may not retain function as part of a bacteriorhodopsin chimeric fusion protein.

Hoflack *et al.* explores the question of whether bacteriorhodopsin is a valid model for studying the three dimensional structures of G protein-coupled receptors, given reported differences in electron diffraction maps of bovine rhodopsin and bacteriorhodopsin. The Hoflack

et al. statement that "bacteriorhodopsin is famous as a template to construct three dimensional models of G-protein coupled receptors" does not provide motivation to replace a portion of the intracellular loop 3 domain with a portion of the intracellular loop 3 region from a G protein-coupled receptor, let alone replacing amino acid residues 171-179 of the intracellular loop 3 domain with a portion of the intracellular loop 3 region from a G protein-coupled receptor.

Okamato *et al.* reported that a soluble synthetic peptide having amino acid residues 259-273 (βIII-2) of β2-adrenergic receptor, which correspond to amino acid residues 50-64 of SEQ ID NO:47), was sufficient to promote GTPγS binding to G_S. There is no suggestion in Okamato to develop a chimeric bacteriorhodopsin-GPCR according to the claimed invention. Applicants respectfully disagree with the Examiner's assertion that Okamato provides motivation by teaching "the need for further study of the structure and function of the third intracellular loop of the human β-adrenergic receptor as is well appreciated in the art, e.g. see Introduction and Discussion."

In the Introduction, Okamato discusses previous efforts to elucidate the region of β2-adrenergic receptor responsible for G-protein coupling, and sets forth the goals of research reported therein, specifically, to identify the region that activates adenylyl cyclase and to investigate the phosphorylation-dependent regulation of that region. Based on the results of that research, Okamato concluded in the Discussion that 'The βIII-2 sequence is expected to be a useful probe for investigating the coupling mechanisms between βAR and various G proteins and the mechanism of PKA action on their coupling.... Thus, this peptide provides a focal point for studying the mechanism of ligand-dependent receptor regulation...' (page 728, column 2, emphasis added). Applicants submit that Okamato, which reports the use of a simple 14-amino acid long βIII-2 peptide to study the activity βAR, does not provide motivation to make the claimed chimeric bacteriorhodopsin GPCR protein. Although Okamato may provide motivation to further study this particular region of βAR, Okamato does not provide motivation to accomplish that objective by modifying Popot, Hoflack, and Teufel. In fact, Okamato suggests that the goal of further characterizing the βAR could be accomplished using the relatively simple βIII-2 sequence as a probe.

In view of the foregoing, Applicants respectfully request that the rejections under 35 U.S.C. 103(a) be withdrawn.

As the application is now in condition for allowance, Applicants request reconsideration of the claims on the merits of the application, withdrawal of the outstanding rejections, and allowance of the claims.

Check number 46474 in the amount of $55.00 is enclosed to cover the fee for the one-

extension of time. No other fee is believed owing in connection with this submission. If a fee is owed, please charge such fee to Deposit Account No. 50-0842.

Should Examiner Brannock feel that any other point requires consideration or that the form of the claims can be improved, he is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

Date: February 3, 2003

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